CARBONYL PERFORMANCE AUDIT

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CAUTION

Disclaimer: This Standard Operating Procedure has been developed for use by ManTech Environmental Technology, Inc. in support of the National Performance Audit Program (NPAP) under contract to the U.S. Environmental Protection Agency and may not be applicable to the activities of other organizations.

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Effective: When approved

CONTENTS

PROCEDURAL SECTION ***Page***

FIGURES

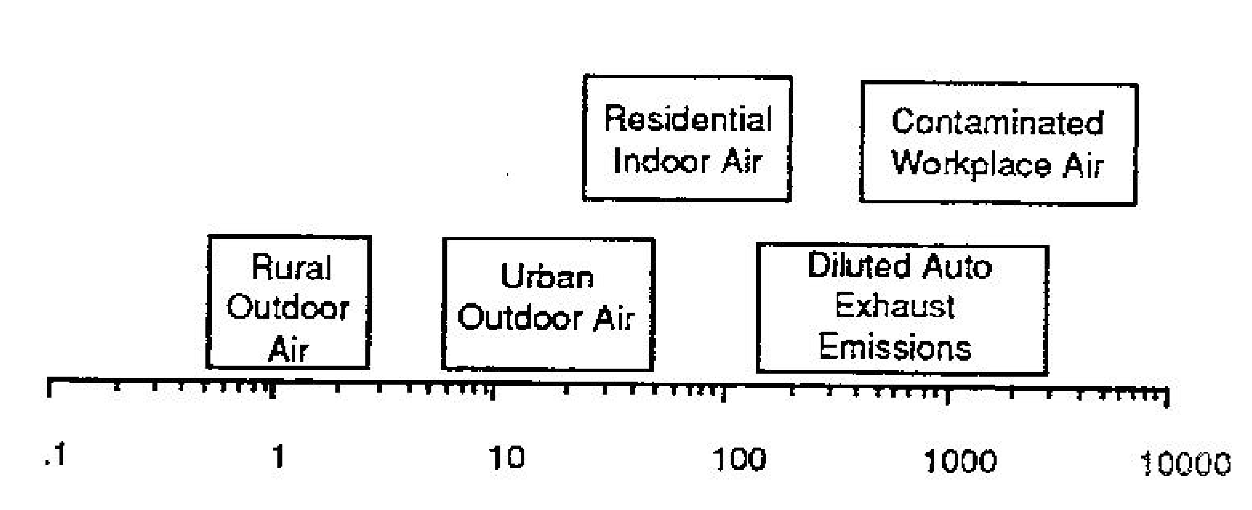
Page

PROCEDURAL SECTION

1.0 Scope & Application

1.1 Aldehydes and ketones are receiving increased attention both as hazardous substances and as promoters in the photochemical formation of ozone in the atmosphere.

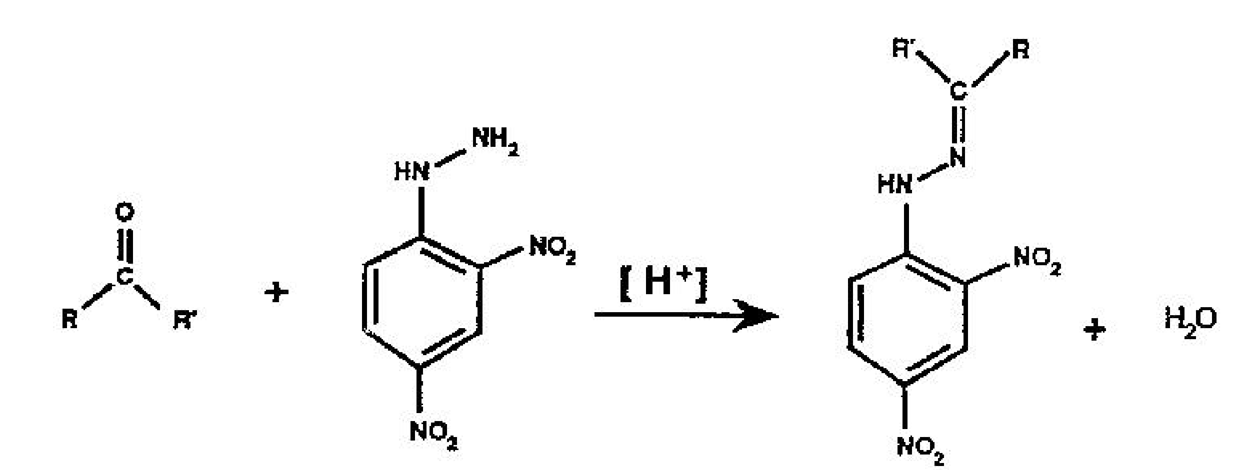
Figure 1 Ranges of Formaldehyde Concentrations in Air, ppbV[5]



1.2 They enter the atmosphere in the exhaust of motor vehicles and other equipment using hydrocarbon and alcohol fuels, as well as other combustion and industrial processes.

1.3 Formaldehyde, the most prevalent aldehyde, is widely used as a preservative, a textile-treatment agent, and an intermediate in the manufacture of urea-formaldehyde and phenol-formaldehyde resins. Figure 1[1,2] shows the ranges of formaldehyde concentrations in several environments.

1.4 In 1986 S. B. Tejada[2] reported the use of cartridges impregnated with 2,4-Dinitrophenyl-hydrazine (DNPH). These cartridges trap the compounds by reaction of the carbonyl group within the cartridge to form stable hydrazone derivatives. This derivatization reaction shown in Figure 2 takes place during sample collection. HPLC analysis[3] of the



Carbonyl Compound 2,4-Dinitrophenylhydrazine (DNPH) DNPH Derivative (a hydrazone)

Figure 2 Derivatization Reaction[5]

cartridge eluates is used to validate the quality of the audit samples. The DNPH cartridge method has become the method of choice for sampling and analyzing aldehydes and ketones in air[4].

1.5 While Method TO-11A gives instructions for preparation of the DNPH cartridges, commercial cartridges are available (Waters Sep-Pak® DNPH-Silica cartridges). The commercial cartridges have the advantage of uniformity, low background, and convenience and are recommended for audit purposes.

1.6 Audit cartridges are loaded with known quantities of carbonyl-DNPH derivatives in the laboratory to conduct PAMS carbonyl performance audits. The audits are conducted to provide quality assurance and instrument performance verification.

2.0 Summary of Method

2.1 This Standard Operating Procedure (SOP) describes ManTech Environmental Technology, Inc. (ManTech) functions in support of the Carbonyl (DNPH) portion of the United States Environmental Protection Agency's (EPA) National Performance Audit Program (NPAP).

2.2 These responsibilities include the preparation and shipment, three times annually from March through September, of derivatized formaldehyde, acetaldehyde, and acetone on Sep-Pak® DNPH-Silica cartridges. Concentrations are specified by the NPAP Project Manager within established limits.

2.3 Typically the spiking levels range from 0.5 to 5 : g/cartridge as the free carbonyl compound. The range is limited by the quantitation limit on the low end of the range. The high end of the range is limited by the cartridge capacity or approximately 2.5 micro-moles of free carbonyl compound. Each set of audit samples includes a blank cartridge.

2.4 Procedures for each step in the execution of preparing and verifying the audit samples are described in detail. These steps include cleaning glassware, preparing stock solutions, loading cartridges, eluting the prepared cartridges, and analyzing sample extracts.

3.0 **Definitions**

3.1	Aldehyde:	Carbonyl compound with the general formula HR-C=O
3.2	Batch:	All carbonyl cartridges prepared for an audit
3.3	Carbonyl Compound:	Any of a group of oxygen containing compounds with the QC=O functional group present R
3.4	Derivative:	The more stable, less volatile compound resulting from the reaction of an aldehyde or ketone with DNPH
3.5	DNPH:	2,4-Dinitrophenylhydrazine
3.6	Free carbonyl:	Underivitized carbonyl compound
3.7	HPLC:	High Performance Liquid Chromatography
3.8	Ketone:	Carbonyl compound with the general formula R-C=O T
3.9	Ozone:	Triatomic oxygen, O_3, found in the atmosphere in varying

proportions

3.10 Photochemical: Of, relating to, or resulting from the chemical action of radiant energy, especially light

3.11 Primary Stock Solution: A stock solution prepared directly from pure reagent

3.12 Secondary Stock Solution: A stock solution prepared from another stock solution

3.13 Set: Consists of a blank, low level, and high level cartridge

4.0 Health & Safety Warnings

4.1 Standard safety procedures for working with chemicals must be observed at all times.

4.2 Chemical-resistant gloves and safety glasses must be worn while preparing stock solutions and when cleaning glassware.

4.3 Heat-resistant gloves must be worn when removing glassware from the oven.

4.4 Lint-free gloves or chemical-resistant gloves must be worn when spiking cartridges.

4.5 This work must also be done using a ventilation hood.

5.0 Interferences

5.1 Note: Contamination due to exposure of the cartridges to aldehydes and ketones such as formaldehyde and acetone is the most likely source of interference.

5.2 Laboratory air often holds high concentrations of acetone. This problem may be alleviated by removing acetone sources from area where cartridges are handled and/or modifying overall usage of acetone in the laboratory when cartridges are being handled.

5.3 Improperly cleaned glassware and syringes are sources of contamination as are labeling inks, adhesives, and packaging containers.

5.4 In some instances, HPLC grade acetonitrile may contain traces of aldehydes and ketones, especially acetone. An aldehyde or ketone in acetonitrile at a concentration of 10 µg/L adds 0.1 µg of aldehyde or ketone per cartridge to background values. If the solvent is unacceptable for a particular application, the supplier should be contacted and/or the acetonitrile purified[5].

5.5 Interferences from other aldehydes or ketones with the analytical method for formaldehyde, acetaldehyde, and acetone are minimal when the chromatographic resolution requirements are met.

6.0 Apparatus, Reagents & Materials

6.1 Facility: This SOP assumes a laboratory equipped with water, sink, water aspirator, ventilation hood, electricity, and storage cabinet for flammable solvents.

6.2 Apparatus

1. Bag sealer
2. Chemical storage freezer
3. Chemical storage refrigerator
4. Oven, small, preferably a vacuum oven
5 Water aspirator

6.3 Reagents

1. Acetonitrile, HPLC grade with a purity of 99.9%
2. 2,4-Dinitrophenylhydrozone of formaldehyde, purified to $99% (available from Radian, Corp. or synthesized, see Appendix)
3. 2,4-Dinitrophenylhydrozone of acetaldehyde, purified to $99% (available from Radian, Corp. or synthesized, see Appendix)
4. 2,4-Dinitrophenylhydrozone of acetone, purified to $99% (available from Radian, Corp. or synthesized, see Appendix)
5. 2,4-Dinitrophenylhydrozone of other carbonyl compounds as required, purified to $99% (available from Radian, Corp. or synthesized, see Appendix)
6. Methanol, HPLC grade

6.4 Materials

1. Class A volumetric flasks w/stoppers, assorted sizes
   e.g., 5.0, 10.0, 25.0, 50.0, and 100 ml
2. Class A pipets, various sizes
3. Chemically inert refrigerant packs, freezing point -23°C
4. Chemical-resistant gloves
5. Gastight® syringes, Hamilton, various sizes
6. Hamilton Syringe Cleaner, 120 VAC

7. Lint-free Kimwipes®, small box
8. Lint-free gloves
9. Pipet rack and washer
10. Styrofoam® insulated shipping boxes
11. Small Pyrex® beakers
12. Vials with Teflon® lined septum caps
13. Waters Sep-Pak® DNPH-Silica cartridges (Millipore Corporation, Part No. 37500)
14. "Zipper" seal bags (for keeping spiked cartridges together)

7.0 Procedure

7.1 Syringe Cleaning

CAUTION

Using the Hamilton Syringe Cleaner without the water aspirator can result in severe damage to the cleaner.

Note: Clean all syringes prior to use.

1. Plug in the Hamilton syringe cleaner and allow it to heat.
2. Remove the plunger of the syringe, rinse the plunger several times with methanol, and place it in a small clean beaker.
3. Insert the needle of the syringe into the cleaner.
4. Introduce methanol into the barrel of the syringe while pulling a vacuum by means of a water aspirator. Repeat 5 times.
5. Remove the syringe, place it in the beaker (needle end up) with its plunger, air dry, then dry in a 60-65°C oven.
   - The syringe and plunger may be stored in the heated oven until needed.
   - Several syringes may be stored in the same beaker but they must all be of

compatible plunger and barrel size.

7.2 Syringe Performance Verification

1. Check syringes periodically for bent needle tips or physical blockages, such as from a small piece of septum.
2. Visually inspect needles before and after every use. If a bent tip is found, set that syringe aside for performance verification and restoration of the needle tip. Use a file for deburring the tip. Using gloved hands gently straighten bends lower down on the needle.
3. If methanol flows sluggishly through the syringe barrel during the cleaning process and no visible blockage is present, the needle may be partially plugged:
   a. Submerge the needle tip in methanol and gently depress (do NOT force) the plunger. The generation of bubbles indicates no blockage.
   b. If blocked, clean the needle with the syringe cleaning wire (provided with the syringe) according to the manufacturer's directions until bubbling occurs. Reclean the syringe with methanol, as necessary.
   c. Dispose of the syringe appropriately if the blockage remains.

7.3 Glassware Cleaning

1. Rinse all volumetric flasks, ground glass stoppers, pipets, syringes, vials, caps, and septum cap liners several times with acetonitrile.
2. Air dry briefly in the hood.
3. Dry in a 60-65˚C oven for 1 hour.
4. Cap the volumetric flasks and vials immediately after removal from the oven to prevent contamination.

7.4 Preparation of Stock and Standard Solutions

7.4.1 Primary Stock Solutions

Stock solutions may be purchased from standards manufacturers. When purchased from Radian, Corp. the primary stock solutions are shipped in 1.2 ml. ampules of acetonitrile and the concentration is 500 ug/ml as carbonyl.

7.4.2 Secondary Stock Solutions

Note: Fresh secondary standards must be prepared for each batch of cartridges.

1. Allow the reagents, stock solutions, pipets, syringes, volumetric flasks, and other

glassware to equilibrate to room temperature.

2. Calculate the volume of primary stock solution required (see **Section 12.2**).
3. Secondary stock solutions may contain more than one compound.
4. Using a clean, dry pipet or syringe, quantitatively transfer the volume to the volumetric flask.
5. Fill to the mark with acetonitrile, stopper and mix well.
6. Transfer the solution to Teflon® lined septum sealed vials.
7. Label the vials.
8. Unopened vials maybe stored in the refrigerator for up to 1 year.

7.5 <u>Loading Carbonyl Compounds onto DNPH-Silica Cartridges</u>

7.5.1 Prepare each audit from a single lot of cartridges. Record the Lot Number in the Carbonyls Logbook, "Carbonyl Sample Preparation." Prepare performance evaluation cartridges in sets of three consisting of the following:

1. blank cartridge
2. low level cartridge, 0.50-1.50 :g carbonyl compound/cartridge
3. high level cartridge, 2.50-5.00 :g carbonyl compound/cartridge

However the concentration values should vary from audit to audit.

7.5.2 Assign cartridge ID numbers according to the following scheme:

XYZZ

where X = concentration level (1,2,3)
Y = audit of calendar year (1,2,3)
ZZ = year (e.g., 95, 96,...)

e.g., carbonyl sample at level 2, for the third audit of 1995 would have the cartridge ID number of 2395

7.5.3 When all cartridges have been spiked, write a brief report to the Data System Coordinator stating the concentrations and sample numbers prepared and the closing date for the audit. These values are entered in the NPAP data system as "Theoretical."

7.6 Loading the Cartridges with Derivatized Carbonyl Compounds

1. Allow secondary stock solutions, syringes, cartridges, and glassware to equilibrate to room temperature.
2. Rinse the appropriately sized syringe three times with stock solution.
3. Remove the cartridge from its factory sealed bag.
4. Remove both the end cap and plug from the cartridge.
5. Pierce both polyethylene filters (see Figure 3) with a syringe needle.

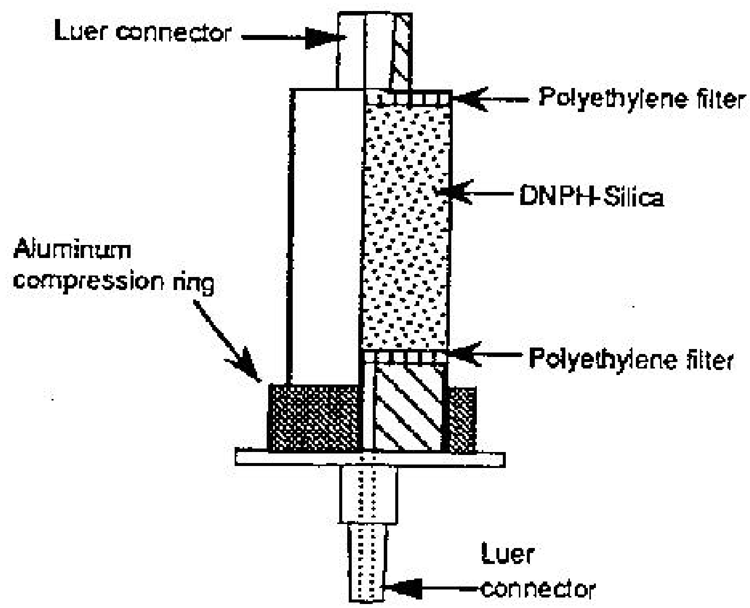


Figure 3 Cutaway View of Sep-Pak® DNPH-Silica Cartridges[5]

6. With the syringe, withdraw a calculated (see **Section 12.3**) volume of stock solution.

7. Through the hole pierced in the upper or plug end of the cartridge, inject the stock solution onto the DNPH-silica bed. Rotating the cartridge helps distribute the solution evenly through the bed.

8. Repeat **Steps 5 through** 7 if more than one stock solution is used to load the set of carbonyl-DNPH compounds requested. Up to three stock solutions may be

necessary to accommodate the concentrations requested.

9. Replace the end cap and plug.
10. Heat seal the cartridge in the factory supplied envelope.
11. Place the heat sealed envelope in the factory supplied bag.
12. Label the bag with the assigned cartridge ID number (see **Section 10.3**).
13. Repeat **Steps 2 through 12** for each cartridge until all cartridges have been loaded.
14. In the laboratory notebook, record stock solution information, volume of solution spiked, quantity spiked, compounds spiked, and any notable events.
15. Use "Zipper" type bags to group cartridge sets.

7.7 <u>Preparation of Spiking Quality Control Samples, if requested</u>

1. Place approximately 9 ml of acetonitrile in each of two 10.0 ml volumetric flasks.
2. Dispense the same amount of stock solution(s) into each flask that was loaded on each cartridge.
3. Fill to the mark with acetonitrile, mix and label as with the level (1,2,3), quality control sample number 1 or 2 (*e.g.*, QC11or QC21).
4. Repeat **Steps 1 through 3 in this section** for each loading level requested.
5. Submit quality control samples for HPLC analysis (see **Section 11.0**).

7.8 <u>Elution of DNPH Silica Cartridges, if requested</u>

1. Allow cartridges reserved for analytical validation and the required glassware to equilibrate to room temperature.
2. Open the cartridge container and remove the cartridge.
3. Remove the cartridge plug and cap. Fasten the cartridge in a holder.
4. Elute with two 5.0 ml portions of acetonitrile, using gravity flow, into a 10.0 ml volumetric flask
5. Fill to the mark with acetonitrile and mix.
6. Transfer to a vial and seal with a Teflon-lined septum cap. Label the vial.
7. Repeat **Steps 2 through 6** in this section for each validation cartridge.
8. Submit eluates for HPLC analysis.

7.9 <u>HPLC Calibration Standards, if requested</u>

1. From independent stock solutions, prepare three to five calibration standards in a range which brackets the cartridge eluates.
2. Supply these calibration standards to the HPLC operator.

7.10 Storage of Spiked DNPH Cartridges

7.10.1 Store the prepared cartridges double sealed in their individual containers at 4°C in a refrigerator.

7.10.2 Store only solvents used in the project in the same refrigerator. Place activated charcoal in the refrigerator to reduce background.

7.11 Analysis & Distribution

Note: Analysis is only performed if requested by EPA.

7.11.1 Analyze and distribute samples as early as possible but no later than 6 weeks after preparation.

7.11.2 Compare the HPLC analysis to the "Theoretical" values.

- Accept the samples if the analytical values are within the EPA determined limits.
- If the values exceed the analytical limits, prepare another batch of samples and submit them for analysis.
- In addition, evaluate the process in attempt to identify the source(s) of sample loss or contamination.

8.0 Calculations

8.1 Diluting primary standard

Calculate the concentration of diluted primary standard (C_2) by equation (1)

$$C_2 \ (\mu g\ carbonyl/ml) = C_1 \ x \ \frac{V_1}{V_{Tot}} \qquad \textbf{(2)}$$

where

C_1 = the concentration of the initial solution,
V_1 = the volume transferred, ml
V_{Tot} = final resulting volume, ml

8.2 Diluting primary standard for specific concentration

If a specific concentration, C_2, is required, rearrange Equation (1) to get

Equation (2).

$$V_1 = \frac{C_2}{C_1} x \; V_{Tot} \qquad \textbf{(3)}$$

Example:

Prepare 10 milliliters of 500 ug/ml formaldehyde from the existing stock solution by placing 4.6 milliliters of the stock into a volumetric flask and fill the flask to the line with acetonitrile.

$$V_1 = \frac{500 \; ug/ml}{1071.6 \; ug/ml} \; x \; 10 \; ml = 4.6 \; ml$$

8.3 Concentration to Amount on Cartridges

One hundred :l of spiking solution is a convenient amount of solution to place on a cartridge with a 1.0 ml holdup volume. Use Equation (3) to calculate the concentration of the spiking solution required to achieve the requested spiking level in :g of free carbonyl/cartridge.

$$C_2 \; (\mu g/ml) = \frac{Wt \; per \; Cartridge \; (\mu g)}{100 \; (\mu l)} \; x \; 1000 \; (\mu l/ml) \qquad \textbf{(4)}$$

Example:

The concentration of spiking solution required to spike 5 ug of carbonyl using 100 ul of solution is 50 ug/ml.

$$C_2 = \frac{5 \; ug}{100 \; ul} \; x \; 1000 \; ul/ml = 50 \; ug/ml$$

8.4 Volume to Amount on Cartridge

Where the concentration of the spiking solution can not be adjusted by dilution, the volume placed on the cartridge may be adjusted to achieve the requested spiking level. Equation (5) shows the calculation of the spiking volume, V_{spike}. The spiking volume should not be less than 10 :l nor more than 200 :l. Choose a syringe that will deliver the volume in one filling and uses at least half of its capacity.

$$V_{spike}\ (\mu l) = \frac{Wt\ per\ Cartridge\ (\mu g)}{C_2\ (\mu g/ml)}\ x\ \ 1000\ (\mu l/ml) \qquad \textbf{(5)}$$

Example:

One hundred milliliters of spiking solution is required to add 5 ug of carbonyl to the cartridge when the concentration of the solution is 50 ug/ml.

$$V_{Spike} = \frac{5\ ug}{50\ ug/ml}\ x\ \ 1000\ ul/ml = 100\ ul$$

9.0 <u>Data Management</u>

9.1 Participant Data. Participant audit data is sent directly to the Data Entry personnel and handled according to NPAP-SOP-005: Computer Data Entry, Report Printing, and System Maintenance for the NPAP.

9.2 Record all laboratory data and comments in the Carbonyl Preparation Logbook.**QUALITY CONTROL/QUALITY ASSURANCE SECTION**

1.0 The acceptable accuracy of the analytical validation is set by the performance acceptance range for the audit participants. Currently this range varies with compound and the nominal amount on the cartridge. The precision of the HPLC method is acceptable if the standard deviation of three or more replicates <5% of the mean in the concentration range 10-fold or more above the detection limit.

2.0 The same lots of cartridges and reagents are used for the exposure and solvent blanks as are used for the audit sets.

2.1 **<u>Exposure Blanks</u>**

- For each audit, six cartridges are selected from the current lot as exposure blanks.
- These cartridges are handled as the treatment group except no stock solutions are added.

2.2 **<u>Solvent Blanks</u>**

- For each audit,six cartridges are selected from the current lost as solvent blanks. - Each of these cartridges is spiked with the same volume of acetonitrile as was the volume of the sample spike.

2.3 Spiked Cartridges

- For each audit, six cartridges are selected from each concentration level prepared for an audit.
- These cartridges are selected from the beginning, middle and end of the loading sequence for a level.

3.0 Sample Archiving

- For each audit, a minimum of two sets of cartridges are retained.
- These sets may be released for shipment to participants who request audit materials after regular shipment has been made or other purposes requested by the Project Manager.

4.0 The HPLC analysis is compared to the "Theoretical" values. The analytical values must be within the EPA determined limits for the samples to be accepted.

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